Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplementary Appendix

Table of contents

PCR Testing

PCR testing was performed by the National Biocentre in Milton Keynes, which processes approximately 20% of samples from the whole are home testing programme, using an Applied Biosytems 7500 Fast real time PCR system and using the Applied Biosystems TaqPath™ 1-Step Multiplex Master Mix (No ROX) (Cat. A28523) and TaqPath COVID-19-ASY-KIT 1000 (Cat. A47817). Primer sequence details are not currently published. Quantification of the Positive/Negative/Inconclusive status of each sample is determined using the UgenTec FastFinder Interpretative software.

Ethics

This was an observational surveillance study carried out under the permissions granted under regulation 3(4) of The Health Service (Control of Patient Information) Regulations 2020. [1]

Table S1. Median (IQR) Ct value* by week by PCR gene target[#]

	5-12 Oct	12-19 Oct	19-26 Oct	26 Oct-2 Nov	2-9 Nov	9-16 Nov	16-23 Nov	23-30 Nov	30 Nov-7 Dec	7-14 Dec	14-17 Dec
One gene	34.2 (33.4-35.0)	33.4 (32.6-35.0)	33.1 (32.6-33.8)	33.4 (32.4-34.2)	33.5 (32.6-34.2)	33.7 (33.2-34.6)	34.5 (33.9-35.4)	32.7 (32.2-34.4)	33.5 (32.3-34.4)	33.1 (30.9-33.3)	NA
N + ORF	32.9 (32.0-34.1)	32.5 (31.3-33.6)	23.4 (21.8-32.1)	31.3 (20.6-32.8)	26.4 (20.6-31.9)	28.1 (19.4-33.2)	22.5 (18.0-29.5)	22.6 (17.4-29.8)	21.0 (17.3-26.4)	20.4 (16.9-24.9)	20.0 (15.7-23.4)
Three genes	22.4 (18.2-27.9)	23.4 (19.4-27.2)	21.0 (18.6-25.4)	22.2 (19.6-25.8)	22.6 (19.1-26.3)	21.3 (18.5-24.9)	22.7 (19.2-26.9)	22.2 (19.2-27.6)	23.0 (19.6-27.0)	22.5 (19.2-27.4)	22.4 (20.3-25.2)
Overall	27.3 (20.5-32.9)	26.1 (21.0-32.4)	21.8 (18.9-26.5)	23.2 (19.6-28.8)	23.0 (19.4-27.3)	22.3 (18.5-27.4)	23.7 (19.6-29.9)	22.8 (18.6-28.7)	22.9 (19.0-27.3)	20.9 (17.7-26.3)	20.8 (16.7-24.6)

*The highest Ct value of the detected gene targets was used to calculate the median Ct value by week.

Cross-sectional surveys during periods of increasing incidence may overestimate changes in Ct value over time, as the ratio of incident to prevalent cases changes. [2] This effect is reduced by national policy recommendations which advise that individuals should not be retested in the 90 days following a positive test.

Figure S2. Proportion of positive tests associated with new variant by week*, stratified by region # (05/10/20 – 17/12/20) §
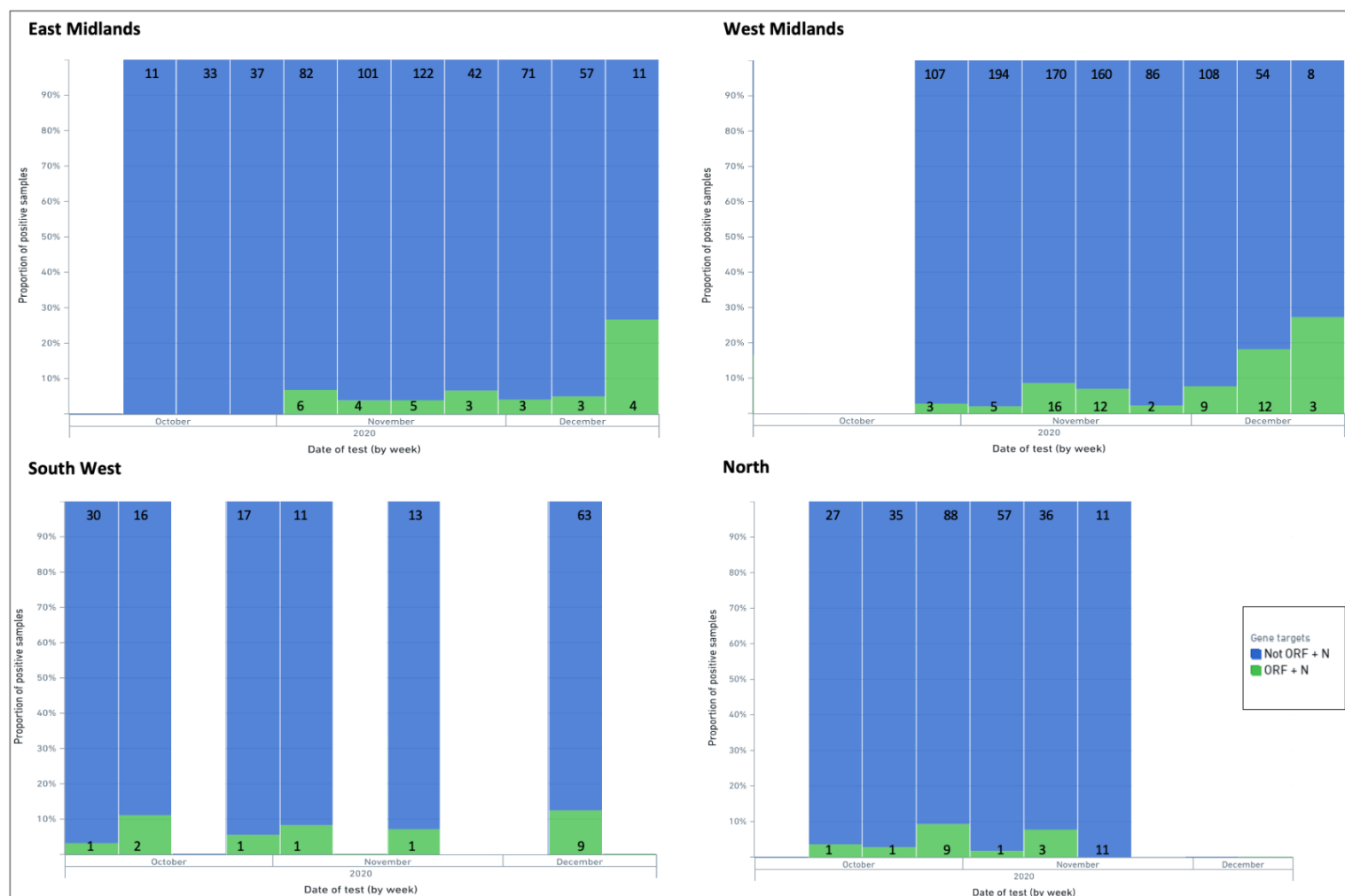
*The number of PCR positive samples per week for each ORF + N, and not ORF + N samples is listed in each bar. Only positive samples from weeks with > 10 cases have been presented. # Samples from Yorkshire and Humber, the North West and the North East were analysed as a single group labelled "North" due to the small number of samples from each region and subsequent risk of deductive disclosure. § Confidence intervals for proportions have not been calculated as we were unable to adjust for clustering of samples at the level of the individual or at the level of the LTCF because this information was not available at the time of analysis. National testing recommendations suggest that LTCF staff or residents should not undergo repeat nasopharyngeal sampling for 90 days after a positive test.

Figure S3. Proportion of positive tests associated with the new variant by week, stratified by age group (05/10/20 – 17/12/20)
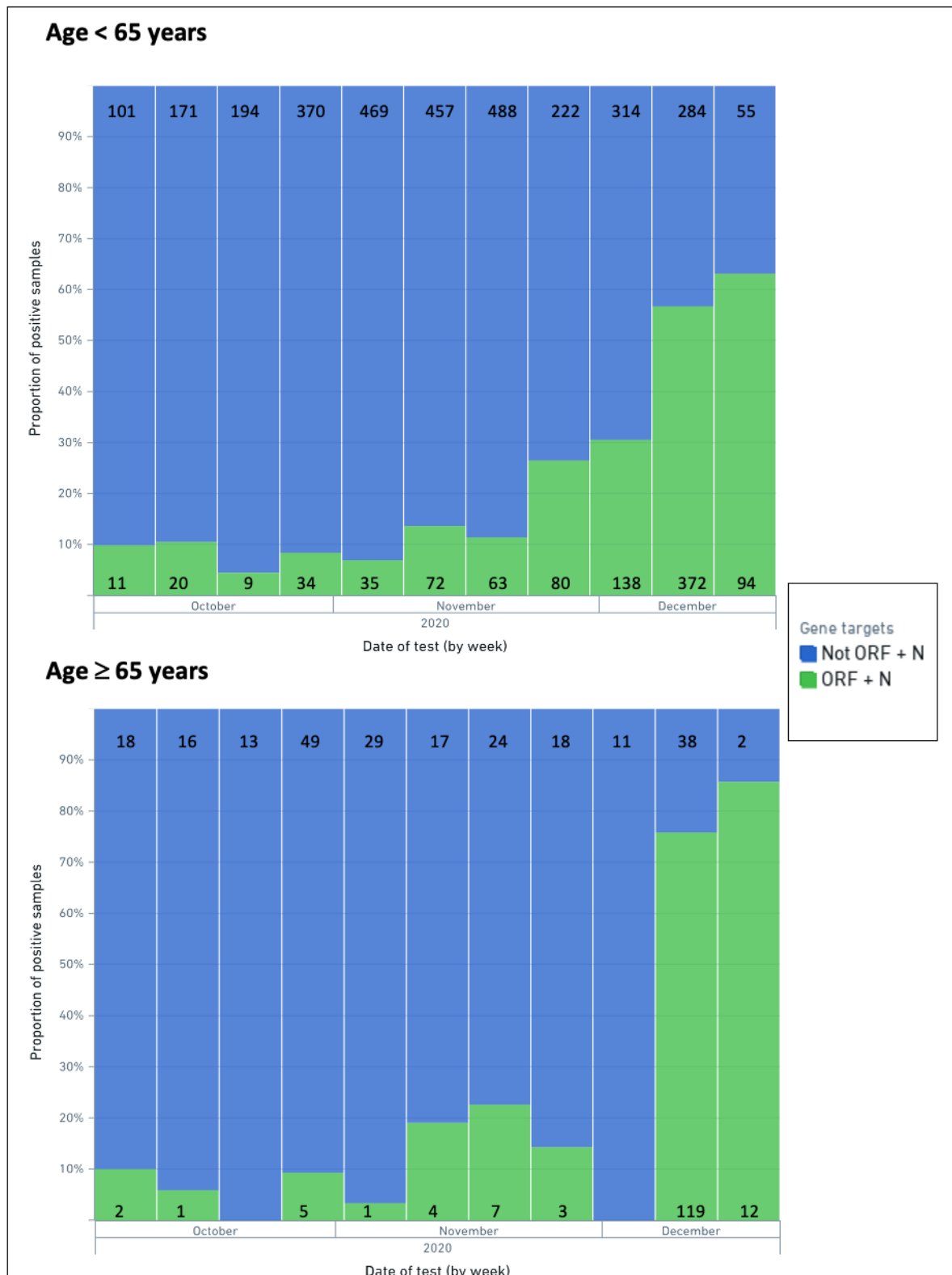
Sensitivity analysis

As the Ct is a good indicator of the viral load in a sample, it has been suggested that these values may correlate with severity of infection. [3,4] In addition, the probability of culturing live virus from samples drops significantly as the Ct rises above 30. [5]

In order to ensure that the absence of detectable S gene in the samples that we analysed was not reflective of low infectiousness of the viral isolates but represented genetic variation, we restricted our analysis to only include gene targets that were detected at a Ct < 30 (Figure S4). The same pattern of rapid emergence of the variant from mid-November onwards was seen when the analysis was restricted to analysis of samples with gene Ct values < 30 and stratified by region or by age group (data not shown).

Figure S4. Proportion of samples with S gene dropout by week, showing the impact of excluding samples with gene Ct values > 30 ((05/10/20 – 17/12/20)*
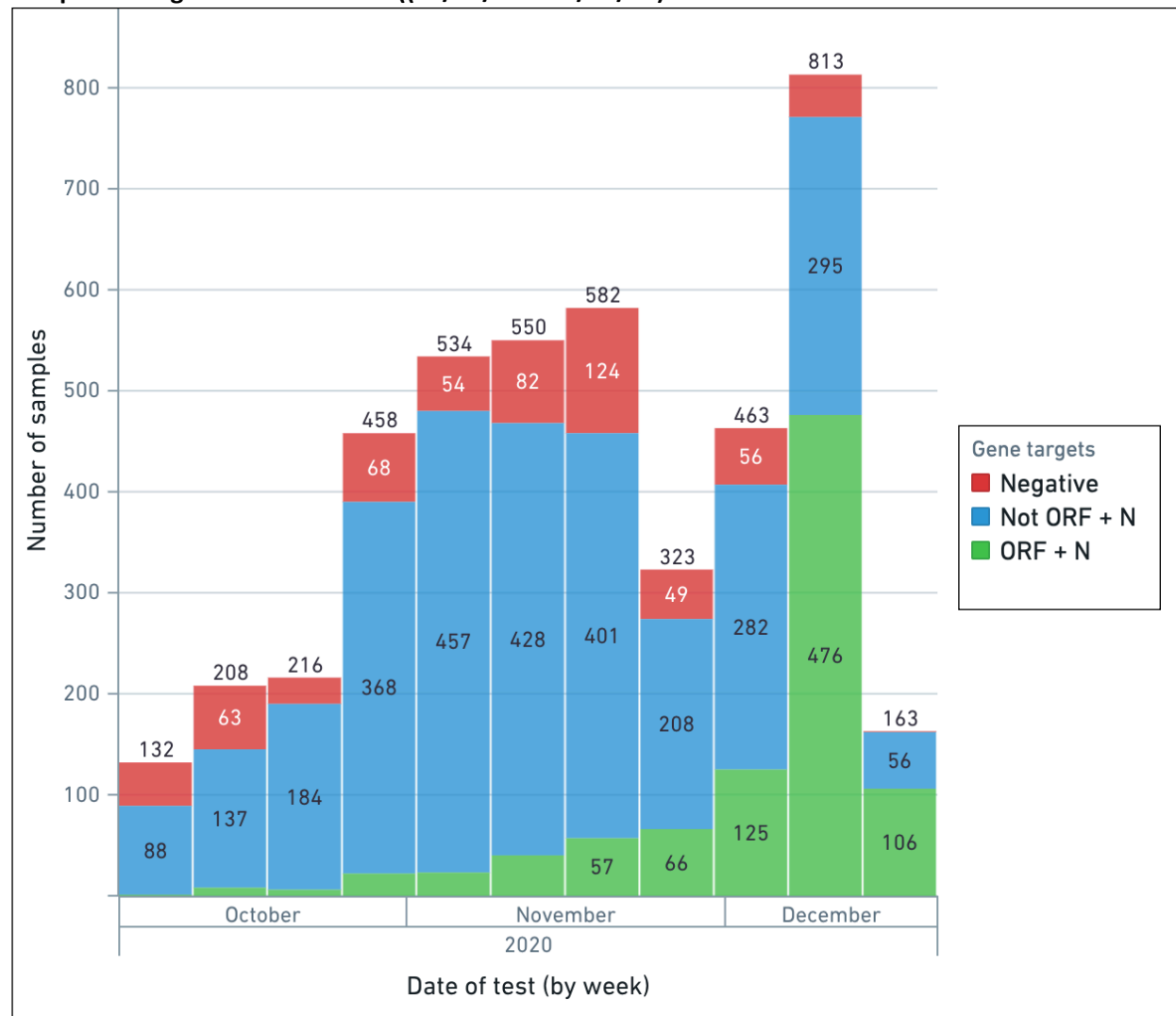
*Samples have been re-classified as negative if all three gene targets have been detected at Ct ≥30

References

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